The signature of scrapie

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The signature of scrapie: differences in the PrP genotype profile of scrapie-affected and scrapie-free UK sheep flocks

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The amino-acid sequence of the PrP protein plays an important role in determining whether sheep are susceptible to scrapie. Although the genetics of scrapie susceptibility are now well understood, there have been few studies of the PrP gene at the population level, especially in commercially farmed sheep. Here we describe the PrP genetic profiles of the breeding stock of four UK sheep flocks, comprising nearly 650 animals in total. Two flocks had been scrapie affected for about eight years and two were scrapie free. Scrapie-resistant PrP genotypes predominated in all flocks but highly susceptible genotypes were present in each case. The distribution of PrP genotypes was similar in the scrapie-affected and scrapie-free flocks. The former, however, showed a slight but significant skew towards more susceptible genotypes despite their previous losses of susceptible sheep. Surprisingly, this skew was apparent in younger, but not older, sheep. We suggest that these patterns may occur if sheep flocks destined to become scrapie affected are predisposed by a genetic profile skewed towards susceptibility. The age structure of the scrapie-affected flocks suggests that the number of losses attributable directly or indirectly to scrapie considerably exceeds that recognized by the farmers, and also that significant losses may occur even in sheep of a moderately susceptible genotype. Similar patterns were not detected in the scrapie-free flocks, indicating that these losses are associated with scrapie infection as well as genotype.

Keywords: scrapie; PrP; genotype; sheep

1. INTRODUCTION

Scrapie is a transmissible neurodegenerative disease of sheep and goats characterized by changes in behaviour, trembling, pruritus and incoordination, proceeding to recumbency and death. Illness usually lasts one to two months (Dickinson 1976) and the majority of infected animals die at between two and four years of age (Detwiler 1992). The brains of affected animals have vacuolated neurons and an abnormal form of a host protein called PrP (Van Keulen *et al*. 1995; Foster *et al*. 1996; Caughey & Chesebro 1997).

Polymorphisms in the amino-acid sequence of PrP play a significant role in determining whether individual sheep are susceptible or resistant to scrapie following exposure. Many polymorphisms have been identified to date but three in particular are strongly linked to the occurrence of both experimental and natural scrapie. These are valine (V) or alanine (A) at codon 136, arginine (R) or histidine (H) at codon 154 and glutamine (Q), arginine (R) or histidine (H) at codon 171 (for a review, see Hunter 1997*a*). Out of 12 possible alleles, only five are commonly seen: $A_{136}R_{154}R_{171}$, $A_{136}R_{154}Q_{171}$, $V_{136}R_{154}Q_{171}$, $A_{136}H_{154}Q_{171}$ and $A_{136}R_{154}H_{171}$ (Belt *et al*. 1995). The $A_{136}R_{154}R_{171}$ allele is clearly associated with resistance to scrapie and $V_{136}R_{154}Q_{171}$ is clearly associated with susceptibility (Goldmann *et al*. 1994; Belt *et al*. 1995; Hunter *et al*. 1996). Thus, sheep of genotype $AA_{136}RR_{154}RR_{171}$ appear to be resistant to scrapie (worldwide, only one case in such an animal is known; Ikeda *et al*. 1995), while sheep of the $VV_{136}RR_{154}QQ_{171}$ genotype are highly susceptible (Belt *et al*. 1995; Hunter *et al*. 1996). Some cases of scrapie are observed in sheep of the $AA_{136}RR_{154}QQ_{171}$ genotype, and in certain breeds (e.g. Suffolks) where $V_{136}R_{154}Q_{171}$ is extremely rare this is the most common genotype of scrapie cases (Hunter *et al*. 1997). The $A_{136}H_{154}Q_{171}$ allele may be associated with resistance in some breeds but not in others and the $A_{136}R_{154}H_{171}$ allele may be neutral (Dawson *et al*. 1998).

In a recent anonymous postal survey of sheep farmers in the UK, 85% of respondents reported never having had scrapie in their flocks (Hoinville *et al*. 1999). In the light of the genetics of scrapie susceptibility, this raises an important question: Do flocks without scrapie have very few, or no, animals of susceptible genotype or have they simply avoided exposure to challenge?

As the sheep that die of scrapie are of certain genotypes and above a certain age, the genotype profile of young animals on a scrapie-affected farm is expected to differ from that of older animals. Specifically, on a scrapie-affected farm a lower frequency of susceptible genotypes is expected in older sheep as some of the susceptible animals will have died. Examination of the age–genotype profile of an entire flock allows further questions to be addressed: Is the incidence of scrapie in affected flocks sufficiently high to change the genotype profile of older sheep by a detectable amount? And most importantly, does the number of losses estimated from the age structure agree with the number of scrapie cases noted by the farmer? This is pertinent, as scrapie-affected farms report having more sheep that die of unknown causes than scrapie-free farms (McLean *et al*. 1999) and a high proportion of sheep that die from unknown causes may, in

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fact, show signs of scrapie when examined using histopathology (Clark & Moar 1992; Clark *et al*. 1994). The incidence of scrapie infection may, therefore, be considerably greater than that estimated from clinical cases.

We are starting to answer these questions by undertaking a large-scale survey of the genotypes of animals in scrapie-affected and scrapie-free sheep flocks in the UK. Here we report initial findings based on nearly 650 PrP genotypes obtained from four flocks, two with scrapie and two scrapie free.

2. MATERIAL AND METHODS

Sheep farmers willing to participate in this study were recruited using advertising, distribution of publicity documents and attendance at sheep events. For each scrapie-affected flock accepted into the study, a scrapie-free flock with similar numbers of the same breed was randomly selected from a pool of potential matches. All farmers were offered free genotyping of their entire breeding stock in return for access to their sheep and relevant information. Farmers that volunteered to participate in our study were probably more familiar with the genetics of scrapie than the 'average' sheep farmer. However, there was no evidence of such a difference between the volunteers with and without scrapie-affected flocks.

Participation in the study is confidential and, to preserve anonymity, we are unable to name sheep breeds. The flocks considered here are pedigree and of two breeds, both of which are known to have the same three PrP alleles ($A_{136}R_{154}R_{171}$, $A_{136}R_{154}Q_{171}$ and $V_{136}R_{154}Q_{171}$) and are considered to have similar associations between PrP genotype and scrapie risk (Dawson *et al*. 1998). All sheep were expected to have arginine (R) at codon 154 and we therefore analysed polymorphic codons 136 (alanine (A), or valine (V)) and 171 (glutamine (Q), arginine (R) or histidine (H)) only and obtained six genotypes: $AA_{136}RR_{171}$, $AA_{136}QR_{171}$, $AA_{136}QQ_{171}$, $VA_{136}QR_{171}$, $VA_{136}QQ_{171}$ and $VV_{136}QQ_{171}$

Each farm was visited once and blood samples were taken from every animal in the pedigree flocks. The numbers of samples taken were 73 (breed 1, scrapie affected), 71 (breed 1, scrapie free), 189 (breed 2, scrapie affected) and 312 (breed 2, scrapie free). Both scrapie-affected flocks noted their first cases of scrapie in 1990 and reported incidences of 1.5 (breed 1, *ca*. 2%) and 3 (breed 2, *ca*. 1.6%) cases per year on average. In the latter flock, scrapie has been confirmed in three sheep of known genotype: two $VA_{136}RR_{154}QQ_{171}$ and one $VV_{136}RR_{154}QQ_{171}$. Equivalent information is not available for the other scrapie-affected flock. Scrapie has never been observed in the two scrapie-free flocks.

For each sheep, approximately 5 ml of blood was collected into an EDTA-vacutainer and stored at −20 °C prior to genotype analysis. Genotype analysis was performed either by restriction digestion and differential hybridization as described in Hunter *et al*. (1993, 1996) or by sequencing using an ABI Prism 377 DNA sequencer as recommended by the manufacturer (PE Applied Biosystems, Warrington, UK). In short, DNA was isolated from 100–500 µl of blood using a Nucleon DNA extraction kit (Anachem, Luton, UK) and then subjected to 30 cycles of PCR amplification with oligonucleotide pair 313 GTCAAGGTGGTAGCCACAGTCA and 316 GCTCCACCACTCGCTCCATTATC or pair 314 GGTGAAAAGCCACATAGGCAGT and 315 ACTACAGGGCTGCAGGTAGACA (Goldmann *et al*. 1994). Following purification over Microcon columns (Amicon, Watford, UK) about one-fifth of the PCR product was sequenced with oligonucleotide 4142 TGGAACAAGCCCAGTAAGCC or 9612 GGTGAAGTTCTCCCCCTTGGT using a BigDye™ terminator kit (PE Applied Biosystems). Sequence reactions were purified with isopropanol precipitations and run on 36 cm polyacrylamide gels. Other samples were analysed by Rosgen Ltd (Roslin, UK).

Table 1. *Frequencies of six genotypes found in four breeding flocks of sheep*

(Numbers are percentages of the total flock, n = total flock size.)

genotype	breed 1, scrapie affected ($n = 73$)	breed 2, scrapie affected ($n = 189$)	breed 1, scrapie free ($n = 71$)	breed 2, scrapie free ($n = 312$)
$AA_{136}RR_{171}$	17.8	10.1	33.8	21.8
$AA_{136}QR_{171}$	43.8	30.7	45.1	34.0
$AA_{136}QQ_{171}$	12.3	21.2	15.5	23.1
$VA_{136}QR_{171}$	17.8	18.0	1.4	9.9
$VA_{136}QQ_{171}$	5.5	16.9	4.2	9.9
$VV_{136}QQ_{171}$	2.7	3.2	0.0	1.3

(a) *Data analysis*

Sheep ranged from zero to seven years old and, therefore, there are 48 possible age–genotype categories. To reduce the number of categories with zero counts and to reduce some of the variation between farms, data were aggregated in various ways. In most analyses, data were combined for the two scrapie-affected flocks and the two scrapie-free flocks. The majority of scrapie cases occurred in animals of two to four years of age and, where appropriate, sheep were divided into two age groups: zero to three years old, a young population likely to have been little affected by scrapie, and four to seven years old, an older population likely to have been more affected by scrapie. In some analyses genotypes were combined into three groups: $AA_{136}RR_{171}$ and $AA_{136}QR_{171}$ (no or low risk of scrapie); $AA_{136}QQ_{171}$ and $VA_{136}QR_{171}$ (moderate risk of scrapie); $VA_{136}QQ_{171}$ and $VV_{136}QQ_{171}$ (high risk of scrapie). These groups correspond to the risk groups R1–R3, R4 and R5 of Dawson *et al*. (1998). It should be noted that the level of risk associated with a specific genotype may be affected by the breed of sheep and/or the strain of scrapie (Hunter 1997*b*), and the risk groups of Dawson *et al*. (1998) are not necessarily appropriate for all scrapie-affected flocks. From the limited information available, however, scrapie in at least one of the flocks considered here is associated with the high-risk PrP genotypes, suggesting that these groupings are appropriate for our flocks. Finally, our analyses convincingly support this assertion.

3. RESULTS

All flocks included sheep of all genotypes apart from the smaller of the scrapie-free flocks, which lacked $VV_{136}QQ_{171}$ sheep (table 1). All flocks showed a skew towards more resistant genotypes, $AA_{136}QR_{171}$ being the most and $VV_{136}QQ_{171}$ the least frequent genotypes.

The most resistant genotypes ($AA_{136}RR_{171}$ and $AA_{136}QR_{171}$) were more frequent in the scrapie-free than the scrapie-affected flocks (combined data, figure 1*a*); conversely, the most susceptible genotypes ($VA_{136}QQ_{171}$

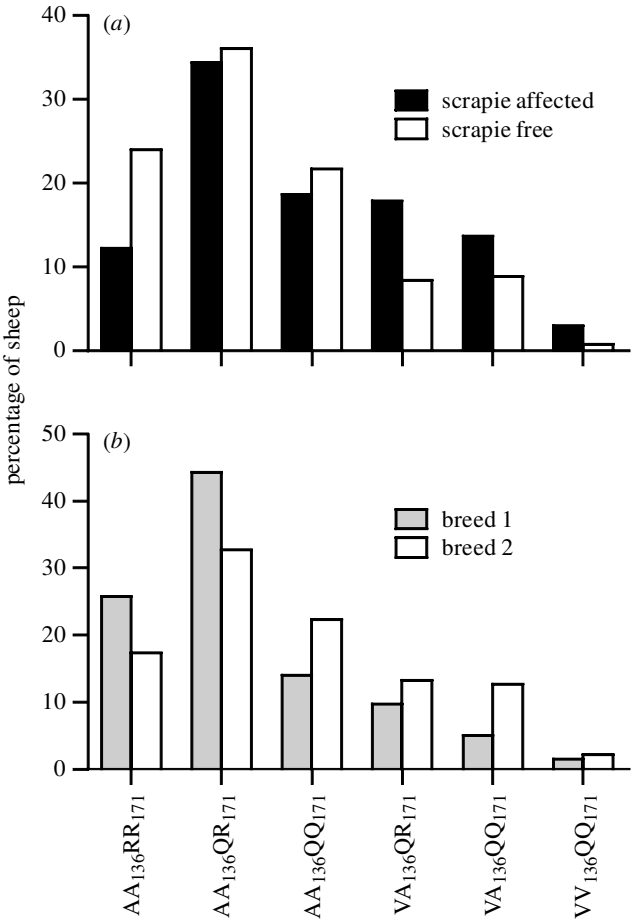

Figure 1. (*a*) Frequencies of six PrP genotypes in scrapie-affected and scrapie-free sheep flocks in the UK. Data are for two scrapie-affected and two scrapie-free flocks combined. Key to genotypes: $AA_{136}RR_{171}$ and $AA_{136}QR_{171}$, no or low risk of scrapie; $AA_{136}QQ_{171}$ and $VA_{136}QR_{171}$, moderate risk of scrapie; $VA_{136}QQ_{171}$ and $VV_{136}QQ_{171}$, high risk of scrapie. (*b*) Frequencies of six PrP genotypes in flocks of two different breeds of sheep. Data are for two flocks of each breed combined.

and $VV_{136}QQ_{171}$) were more frequent in the scrapie-affected flocks. This difference in distribution is significant ($\chi^2 = 30.5$, d.f. = 5, $p < 0.001$) despite the extensive overlap (figure 1*a*).

There was also a significant difference between the genotype frequencies of the two sheep breeds (combined data, figure 1*b*; $\chi^2 = 19.6$, d.f. = 5, $p < 0.001$). The most resistant genotypes ($AA_{136}RR_{171}$ and $AA_{136}QR_{171}$) were more frequent in sheep of breed 1 than breed 2, while more susceptible genotypes were more frequent in breed 2 than breed 1. This result partly confounds the difference between scrapie-affected and scrapie-free flocks mentioned above. As the scrapie-free breed 2 flock was considerably larger than its scrapie-affected counterpart, the genotype profile of the (combined) scrapie-free flocks contains a larger-than-expected number of susceptible genotypes, and this will have tended to lessen the difference between the two flock types. The difference was, nevertheless, significant. If there were no differences in genotype frequency between the two sheep breeds, the observed difference between the scrapie-affected and scrapie-free flocks would have been more significant.

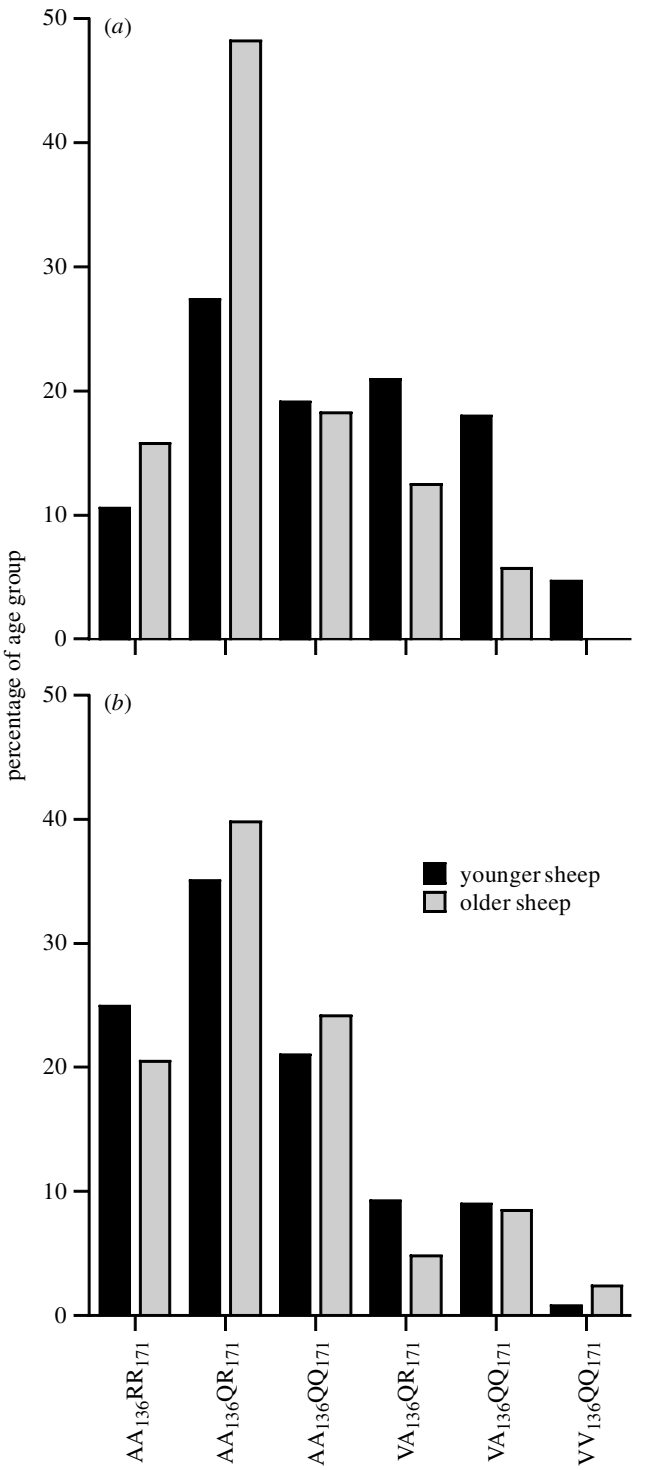

Figure 2. Frequencies of six PrP genotypes in younger sheep (zero to three years old) and older sheep (four to seven years old) in (*a*) scrapie-affected and (*b*) scrapie-free flocks in the UK. Data are for two scrapie-affected and two scrapie-free flocks combined. Key to genotypes: $AA_{136}RR_{171}$ and $AA_{136}QR_{171}$, no or low risk of scrapie; $AA_{136}QQ_{171}$ and $VA_{136}QR_{171}$, moderate risk of scrapie; $VA_{136}QQ_{171}$ and $VV_{136}QQ_{171}$, high risk of scrapie.

In the scrapie-affected flocks the distribution of genotypes in younger (zero to three years old) sheep differed from that in older (four to seven years old) sheep (combined data, figure 2*a*; $\chi^2 = 21.8$, d.f. = 5, $p = 0.001$). Older sheep of more susceptible genotype appear to be

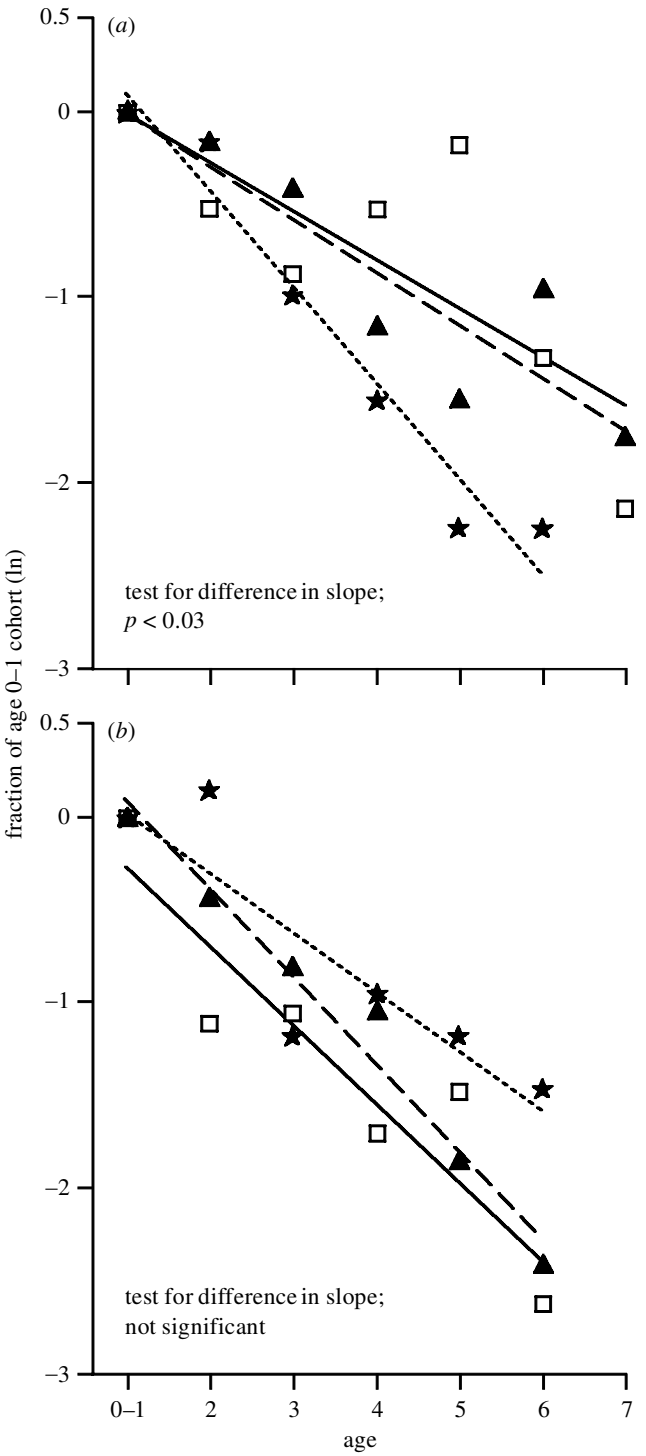

Figure 3. The numbers of sheep of each age present in (*a*) scrapie-affected and (*b*) scrapie-free UK flocks as a fraction of the size of the zero-to-one-year-old cohort. In each figure data are for two flocks combined. All data sets include a point at 0 on the *y*-axis for the age zero-to-one-year cohort. Least-squares regression lines are shown. Sheep were divided according to three PrP genotype categories: open squares and solid line, $AA_{136}RR_{171}$ and $AA_{136}QR_{171}$ (no or low risk of scrapie); solid triangles and dashed line, $AA_{136}QQ_{171}$ and $VA_{136}QR_{171}$ (moderate risk of scrapie); solid stars and dotted line, $VA_{136}QQ_{171}$ and $VV_{136}QQ_{171}$ (high risk of scrapie).

Table 2. *Expected sheep numbers in the scrapie-affected flocks in the absence of any scrapie-associated losses*

(Expected numbers were calculated from the number of younger sheep of the susceptible genotypes and the ratio of older to younger sheep observed in the two most resistant genotypes. The scrapie-associated loss ratio is the ratio of difference to expected.)

	number of older sheep			scrapie-associated
genotype	observed	expected	difference	loss ratio
$AA_{136}QQ_{171}$	16	29	13	0.45
$VA_{136}QR_{171}$	11	32	21	0.66
$VA_{136}QQ_{171}$	5	27	22	0.81
$VV_{136}QQ_{171}$	0	7	7	1

underrepresented. This effect was apparent even when the two most susceptible genotypes ($VA_{136}QQ_{171}$ and $VV_{136}QQ_{171}$) were excluded ($\chi^2 = 8.9$, d.f. = 3, $p < 0.05$). In the scrapie-free flocks, however, there was no difference between the distribution of genotypes in younger and older sheep (combined data, figure 2*b*; $\chi^2 = 4.7$, d.f. = 5, not significant).

This finding suggests that in scrapie-affected flocks there are genotype-specific differences in how sheep numbers decline as their age increases. This is, indeed, the case. In figure 3 the size of each age cohort is expressed as a fraction of the size of the zero to one year old cohort in order to standardize for the different numbers of sheep with each of the six genotypes. As expected, cohort size declines with age because of mortality and the selling of animals. Where mortality (or trade) is greater, however, the rate of decline should be faster. Figure 3 shows that in the scrapie-affected flocks the cohort size of animals of genotypes $AA_{136}RR_{171}$ and $AA_{136}QR_{171}$ (no or low risk of scrapie) declines at a similar rate to that of genotypes $AA_{136}QQ_{171}$ and $VA_{136}QR_{171}$ (moderate risk of scrapie). The cohort size of animals of the most susceptible genotypes ($VA_{136}QQ_{171}$ and $VV_{136}QQ_{171}$), however, declines significantly faster than that of the other genotypes (test for difference in slope, $F_{1,16} = 6.0$, $p < 0.03$). In contrast, in the scrapie-free flocks there were no significant differences in the rates of decline of the different genotype groups. Figure 3 suggests, also, that in scrapie-affected flocks a more rapid rate of decline in the numbers of scrapie-susceptible sheep is apparent in cohorts of four-year-old or older sheep, but not in cohorts of two- or three-year-old sheep.

The previous results suggest that the distribution of genotypes in older rather than younger sheep may differ between scrapie-affected and scrapie-free flocks. In fact, the converse is true. For younger sheep, there was a highly significant difference in the distribution of genotypes between the scrapie-affected and scrapie-free flocks (figure 2, solid bars only, $\chi^2 = 40.1$, d.f. = 5, $p < 0.001$), with the scrapie-free flocks having the greatest skew towards more resistant genotypes. For older sheep there was no difference in the distribution of genotypes (figure 2, shaded bars only, $\chi^2 = 7.5$, d.f. = 5, not significant).

In the scrapie-affected flocks the ratio of older-to-younger sheep for the $AA_{136}RR_{171}$ and $AA_{136}QR_{171}$ genotypes combined is 0.88, a ratio that is, presumably,

unaffected by any losses from scrapie. These flocks have eight young animals of $VV_{136}QQ_{171}$ genotype and, were the age ratio for that genotype also 0.88, seven older animals of this genotype would be expected. In fact there are none, suggesting that seven sheep of the $VV_{136}QQ_{171}$ genotype have been lost for reasons associated with their genotype, death from scrapie being the obvious candidate. We repeated this calculation for the other scrapie-susceptible genotypes (table 2). In all cases the observed number of older sheep is much lower than that expected and, in total, the age structure suggests that there have been approximately 63 losses in scrapie-susceptible genotypes in the two flocks. We repeated this procedure for the two scrapie-free flocks; the total number of losses of the four susceptible genotypes estimated in this way was 0.3 (i.e. not different from zero). The exact number of losses estimated in this manner is affected by the specific age ranges used to define younger or older sheep, but the general pattern is robust for different age ranges. Thus, if sheep become 'old' at age three years rather than four years, then the losses are 57.1 (scrapie-affected flocks) and 4.6 (scrapie-free flocks).

The number of such losses can be expressed as a fraction of the number of older sheep expected in the absence of losses. This fraction, which we call the scrapie-associated loss ratio, indicates the extent to which scrapie infection in a flock affects the numbers of sheep of different genotypes. These ratios are shown in table 2.

4. DISCUSSION

Our results show that sheep of susceptible genotype are found in scrapie-free flocks and pose the question of why scrapie has not occurred. One obvious possibility is that the scrapie-free flocks have not yet been exposed to the agent that causes scrapie, with the implication that should they be exposed in the future scrapie will occur. Furthermore, our results show that when flocks have been infected with scrapie for a number of years (in our study, eight years at the time of sampling), changes occur in the age–genotype profile that are both profound and detectable.

If two sheep flocks initially have similar genotype profiles and one then becomes infected with scrapie, this flock should eventually have a lower frequency of susceptible genotypes because of the deaths of susceptible animals. A difference in genotype frequency should be most apparent in sheep four years old and older, since most scrapie-related deaths will have occurred earlier. In our study a different pattern emerged. While too few flocks have been examined for firm conclusions to be drawn, sheep of susceptible genotype were more frequent in the scrapieaffected flocks, despite the losses from scrapie. Furthermore, the difference in genotype frequency was apparent in the younger, not the older, sheep. These results are consistent with the following suggestion: flocks that are to become scrapie affected have higher frequencies of susceptible sheep (both young and old) than flocks that are to remain scrapie free; losses from scrapie then occur, reducing the numbers of susceptible older sheep; these losses partially 'redress the balance' and the genotype profiles of older sheep in scrapie-affected and scrapie-free flocks are similar, while the genotype profiles of younger sheep remain dissimilar. Under this scenario, sheep flocks destined to become scrapie affected are predisposed by a genetic profile skewed towards susceptibility.

With sufficient replication of farms, differences in the genotype profiles of older animals may become apparent. In particular, in scrapie-affected flocks the frequency of older sheep of the $VV_{136}QQ_{171}$ genotype is expected to be very low because of the extreme level of susceptibility, while in scrapie-free flocks a small but greater frequency of this genotype is expected.

In scrapie-affected flocks the ratio of older-to-younger animals differed with genotype, and this is expected since scrapie affects certain genotypes only. In order to get the observed ratios, however, the number of sheep of susceptible genotype that were lost was estimated to be about 60 in total. This contrasts with the farmers' recollection of 4.5 cases per year (total for the two flocks). The oldest sheep on these farms were seven years old. If we assume that all cases of scrapie occur in two-year-old sheep, then there are six yearly age cohorts in which losses of 4.5 cases each should be detectable, suggesting that we should have detected 27 scrapie-related losses. How can we reconcile this number with the much larger number estimated from the age structure? First, the farmers may have significantly underestimated the number of cases of scrapie that they have observed. Second, the farmers may have inadvertently sold more sheep of susceptible genotype because, perhaps, of poor performance; this poor performance would, however, have to be linked to scrapie infection since genotype-specific losses were not apparent in the scrapie-free flocks. Third, it is possible that the true incidence of scrapie may be considerably greater than that observed by farmers themselves. A recent postal survey found that scrapie-affected farms in the UK have about twice as many sheep found dead from unknown causes as scrapie-free farms (McLean *et al*. 1999), while in the Shetland Islands 20–50% of such sheep were found to be scrapie positive after histopathological examination of brain tissue (Clark & Moar 1992; Clark *et al*. 1994).

In over 20 flocks of sheep of the so-called 'valine breeds' (which includes the two breeds involved in the present study) that are currently being monitored by the Institute for Animal Health, nearly 90% of confirmed scrapie cases are of $VA_{136}QQ_{171}$ and $VV_{136}QQ_{171}$ genotype (M. Baylis, unpublished data). How can the low frequency of confirmed scrapie in other susceptible genotypes (such as $AA_{136}QQ_{171}$ and $VA_{136}QR_{171}$) in such breeds be reconciled with the apparently substantial number of scrapie-associated losses of these genotypes (table 2) from flocks in the present study? We have no answer to this question at present although one possibility is that the causes of scrapie-associated losses discussed above (poor performance, or dying with scrapie that is not recognized) may target these genotypes in particular.

Our results indicate that when scrapie occurs in a flock over several years, it leaves a signature in the age–genotype profile. This signature, which is shown graphically in figure 4, is the absence of all, or nearly all, older animals of $VV_{136}QQ_{171}$ genotype and, when compared to the resistant genotypes, a very small ratio of old to young animals of $VA_{136}QQ_{171}$ genotype. Furthermore, in the

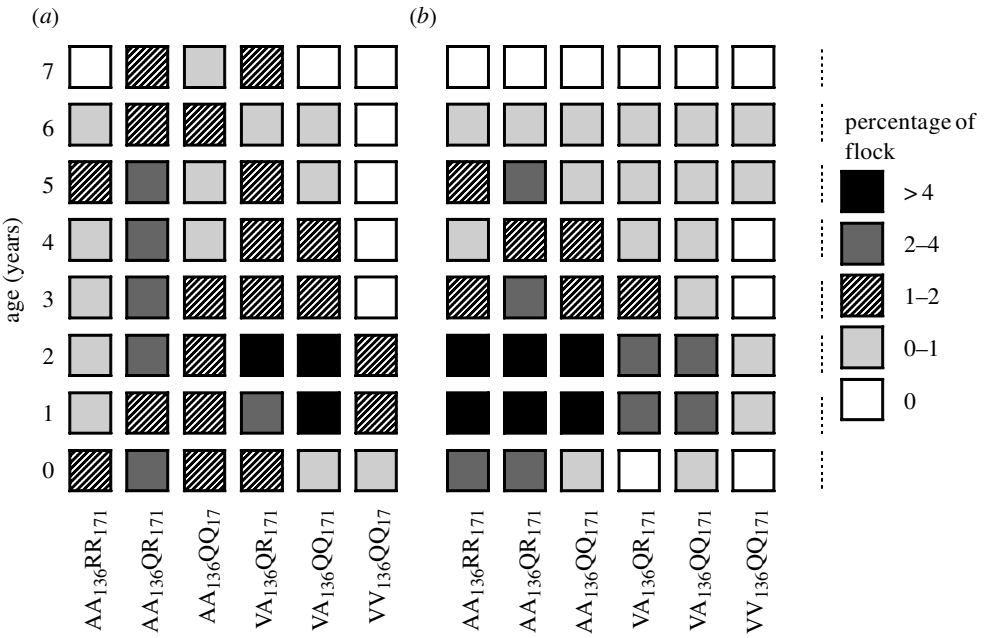

Figure 4. The signature of scrapie. The number of sheep of each age–genotype category, expressed as a percentage of flock size, is shown for (*a*) scrapie-affected and (*b*) scrapie-free flocks. Data for each figure are for two flocks combined. Relative to the most resistant genotypes ($AA_{136}RR_{171}$ and $AA_{136}QR_{171}$), in scrapie-affected flocks there is a rapid decline in the cohort size of the most susceptible genotypes ($VA_{136}QQ_{171}$ and, in particular, $VV_{136}QQ_{171}$).

flocks in our study there is evidence for smaller-than-expected ratios of old to young animals of $AA_{136}QQ_{171}$ and $VA_{136}QR_{171}$ genotypes. The signature left by scrapie may vary according to the scrapie strain and the breed of sheep (and, hence, the genotypes that are targeted in a sheep flock). Less impact will be expected if scrapie has been present in a flock for a relatively short time and, in addition, trade patterns are likely to cause significant amounts of variation. In particular, the genotypes of rams used for breeding will have a large effect on the underlying genotype profile of a flock. Future modelling studies will examine whether such effects can hide the scrapie signature in affected flocks, or whether they can inadvertently suggest a signature in scrapie-free flocks.

The results of a recent anonymous postal survey indicate that more than half of the sheep farms that experience scrapie only see cases in purchased animals (Hoinville *et al.* 1999). It is unknown why the disease does not pass to other animals in the flock. Many farms of this type may use farming practices that do not favour scrapie transmission from purchased to homebred sheep (McLean *et al.* 1999). Another possibility, however, is that the genotype profiles of the challenged sheep flocks may be insufficiently skewed towards susceptible genotypes for a within-flock scrapie epidemic to occur. We are currently investigating the genotype profiles of sheep flocks of this type.

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REFERENCES

Belt, P. B. G. M., Muileman, I. H., Schreuder, B. E. C., Bos-de Ruijter, J., Gielkens, A. L. J. & Smits, M. A. 1995 Identification of five allelic variants of the sheep PrP gene and their association with natural scrapie. *J. Gen. Virol.* **76**, 509–517.

Caughey, B. & Chesebro, B. 1997 Prion protein and the transmissible spongiform encephalopathy. *Trends Cell Biol.* **7**, 56–62.

Clark, A. M. & Moar, J. A. E. 1992 Scrapie: a clinical assessment. *Vet. Rec.* **130**, 377–378.

Clark, A. M., Dawson, M. & Scott, A. C. 1994 Scrapie associated fibrils in found dead sheep. *Vet. Rec.* **134**, 650–651.

Dawson, M., Hoinville, L. J., Hosie, B. D. & Hunter, N. 1998 Guidance on the use of PrP genotyping as an aid to the control of clinical scrapie. *Vet. Rec.* **142**, 623–625.

Detwiler, L. A. 1992 Scrapie. *Rev. Sci. Tech. Off. Int. Epiz.* **11**, 491–537.

Dickinson, A. G. 1976 Scrapie in sheep and goats. In *Slow virus diseases of animals and man* (ed. R. H. Kimberlin), pp. 209–241. Amsterdam: North-Holland Publishing Co.

Foster, J. D., Wilson, M. & Hunter, N. 1996 Immunolocalisation of the prion protein (PrP) in the brains of sheep with scrapie. *Vet. Rec.* **139**, 512–515.

Goldmann, W., Hunter, N., Smith, G., Foster, J. & Hope, J. 1994 PrP genotype and agent effects in scrapie: change in allelic interaction with different isolates of agent in sheep, a natural host of scrapie. *J. Gen. Virol.* **75**, 989–995.

Hoinville, L., McLean, A. R., Hoek, A., Gravenor, M. B. & Wilesmith, J. 1999 Scrapie occurrence in Great Britain. *Vet. Rec.* **145**, 405–406.

Hunter, N. 1997*a* Molecular biology and genetics of scrapie in sheep. In *The genetics of sheep* (ed. L. Piper & A. Ruvinsky), pp. 225–240. Wallingford, UK: CAB International.

Hunter, N. 1997*b* PrP genetics in sheep and the implications for scrapie and BSE. *Trends Microbiol.* **5**, 331–334.

Hunter, N., Goldmann, W., Benson, G., Foster, J. D. & Hope, J. 1993 Swaledale sheep affected by natural scrapie differ significantly in PrP genotype frequencies from healthy sheep and those selected for reduced incidence of scrapie. *J. Gen. Virol.* **74**, 1025–1031.

Hunter, N., Foster, J. D., Goldmann, W., Stear, M. J., Hope, J. & Bostock, C. 1996 Natural scrapie in a closed flock of

Cheviot sheep occurs only in specific PrP genotypes. *Arch. Virol.* **141**, 809–824.

Hunter, N., Goldmann, W., Foster, J. D., Cairns, D. & Smith, G. 1997 Natural scrapie and PrP genotype: case–control studies in British sheep. *Vet. Rec.* **141**, 137–140.

Ikeda, T., Horiuchi, M., Ishiguro, N., Muramatsu, Y., Kai-Uwe, G. D. & Shinagawa, M. 1995 Amino acid polymorphisms of PrP with reference to onset of scrapie in Suffolk and Corriedale sheep in Japan. *J. Gen. Virol.* **76**, 2577–2581.

McLean, A. R., Hoek, A., Hoinville, L. J. & Gravenor, M. B. 1999 Scrapie transmission in Britain: a recipe for a mathematical model. *Proc. R. Soc. Lond.* B **266**, 2531–2538.

Van Keulen, L. J. M., Schreuder, B. E. C., Meloen, R. H., Poelenvandenberg, M., Mooijharkes, G., Vromans, M. E. W. & Langeveld, J. P. M. 1995 Immunohistochemical detection and localization of prion protein in brain-tissue of sheep with natural scrapie. *Vet. Pathol.* **32**, 299–308.

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